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Differences between *Pinus pinea* and *Pinus pinaster* as bioindicators of polycyclic aromatic hydrocarbons

Nuno Ratola^{a,*}, José Manuel Amigo^b, Mónica S.N. Oliveira^c, Rita Araújo^a, José A. Silva^a, Arminda Alves^a

^a LEPAE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

^b Department of Food Science, Quality and Technology, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

^c CEFT, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

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ABSTRACT

The potential of Univariate and Multivariate Analysis and specifically Principal Components Analysis (PCA), has been employed to assess the performance of pine needles as bioindicators of polycyclic aromatic hydrocarbons (PAHs) and particularly emphasize the crucial importance of making a distinction between different pine species if more than one is sampled. Four sampling sessions were done in 29 sites and needles of two common pine species (*Pinus pinaster* Ait. in 19 sites and *Pinus pinea* L. in 12) were collected and analysed using gas chromatography–mass spectrometry (GC–MS) and PCA. The results obtained indicated significant differences between species, attributed to their different morphology. The mean total PAH concentration of the *P. pinaster* needles are over two times higher than *P. pinea*'s. This difference is lower when the results are presented in lipid weight, but still statistically significant. Samples from the two sites with adjacent trees reinforce these conclusions, showing significant differences in terms of PAH mean concentration and aromatic ring patterns.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are atmospheric pollutants which are derived from a broad range of natural and especially anthropogenic sources, such as traffic and industrial activity, through the combustion of oil, wood, and other organic materials and possess high toxicity towards organisms (Solé, 2000). The whole PAH family presents considerable differences in the physical–chemical properties of its elements, which in turn have a decisive influence on their emission, transportation and deposition pathways (Lehndorff and Schwark, 2009).

Biomonitoring has been used as a valid tool to assess the concentration levels of PAHs in the environment since in the late 1980s scientists started considering vegetation routinely as a privileged matrix for the uptake of organic pollutants (Eriksson et al., 1989). The lipid-rich cuticle of plant materials is likely to accumulate such persistent contaminants mainly by atmospheric deposition onto the leaf surface (Simonich and Hites, 1995). Recent approaches even try to establish a quantitative correlation between PAH levels in air and in plants (Zhu et al., 2008). Several plants and trees (evergreen species preferred over deciduous ones) such as grass (Meharg et al., 1998), oak (De Nicola et al., 2005), maple (Wagrowski and Hites, 1997), laurel (Marsili et al., 2001) or olive (Librando et al., 2002)

have been used as bioindicators for polycyclic aromatic hydrocarbons (PAHs) and other pollutants.

Pine trees also proved their effectiveness, especially by the entrapment capacity of their needles (Simonich and Hites, 1995). However, the heterogeneous behavior of plant matrices in general (pine needles from different species in particular) can anticipate diverse entrapment abilities and uptake kinetics (Dunn et al., 1996; Kylin and Sjödin, 2003). Numerous pine species exist all over the world, each one with their own characteristics, and diverse behavior was already reported between some of them (Librando et al., 2002; Piccardo et al., 2005; Ratola et al., 2010a). A substantial variability between several plant species collected in the same site has also been found regarding PAH bioaccumulation (Migaszewski et al., 2002).

In Portugal, pine trees can be found all over the territory, since they are the predominant tree species, representing 33% of the forested area (DGF, 1998). *Pinus pinaster* Ait. and *Pinus pinea* L. are the two most abundant pine species and have been successfully tested before as Portuguese needle samples for the assessment of PAH levels, but focusing on analytical method validation (Ratola et al., 2006, 2009).

One of the main problems in environmental sciences is a huge amount of data generated and the extraction of relevant information from them. This problem has been partially solved in the literature using specific data analysis (restricted to a few sample points, explicit species in a given area, etc.) and applying univariate statistics (e.g. ANOVA tests). Nevertheless, these techniques only

* Corresponding author. Tel.: +351 22 5081400; fax: +351 22 5081449.

E-mail address: nratola@fe.up.pt (N. Ratola).

offer a partial vision of the results and overall perspectives are missed. Multivariate analysis has demonstrated to be the perfect tool to overcome the study of large datasets (Álvarez-Guerra et al., 2010a,b) and to obtain an overall view of general trends in environmental sciences (Terrado et al., 2006; Mas et al., 2010). Of all the multivariate techniques, Principal Component Analysis (PCA) is undoubtedly the most widespread (Kjeller et al., 1991; Tauler et al., 2004; Tsapakis and Stephanou, 2005; Navarro et al., 2006; Dejean et al., 2009; Álvarez-Guerra et al., 2010a,b; Ratola et al., 2010b).

For the current work, four sampling sessions were carried out in 2007 (one for each season) in 29 selected sites which covered a vast area that extends from major urban centers through remote mountain locations in Portugal. In two of them, adjacent trees of both species were sampled. Needles from three different year-classes were collected, since pine trees can preserve the needles of each bud break for several years. Using both univariate statistics and PCA, we tested the hypothesis that there were differences in the behavior of the two pine species regarding PAHs uptake and tried to explain them further by assessing structural and morphological parameters of their respective needles.

2. Materials and methods

2.1. Sampling strategy

The data used in this study was obtained from four sampling sessions (one for each season) carried out in 2007 and described in detail elsewhere (Ratola et al., 2010b). The 29 selected sites comprised the major urban centers in the west coast, some industrial centers, smaller cities in the interior, rural areas and remote locations in the mountains of the Portuguese continental territory (Fig. 1). Needles from *P. pinaster* Ait. or *P. pinea* L. trees (first and second most abundant pine species in Portugal, respectively) were collected, according to their availability in each location. The same trees were samples in all sessions in all sites. In Quintãs (site 27) and Antuã (site 28), it was possible to obtain samples from adjacent trees of both species (Fig. 1). Depending on the species, pine trees can preserve the newly sprung needles for several years. To ensure a proper comparison between both species, only needles from three different year-classes (2005, 2006 and the new needles available from 2007) were considered for this study. Whole needles were removed, preferably from the bottom and outer branches of the trees, placed in sealed plastic bags, kept in the dark and frozen until extraction.

2.2. Chemicals and reagents

Standard mixtures of 16 target PAHs (naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fluo), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenzo[a,h]anthracene (DahA) and benzo[ghi]perylene (BghiP)) and 5 deuterated PAHs (naphthalene- d_8 , acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} and perylene- d_{12}) were purchased (2000 $\mu\text{g ml}^{-1}$) from Supelco (Bellefonte, PA, USA), as well as anthracene- d_{10} (100 $\mu\text{g ml}^{-1}$). International Sorbent Technology (Mid Glamorgan, UK) provided the SPE alumina cartridges (5 g, 25 ml) and hexane and dichloromethane SupraSolv were from Merck (Darmstadt, Germany). Air Liquide (Maia, Portugal) supplied nitrogen for drying (99.995% purity). All glass material was silanised in a 15% dichlorodimethylsilane solution in toluene, both from Aldrich (Milwaukee, WI, USA).

2.3. Characterization of the pine needles

To assess the structural characterization of the *P. pinaster* and *P. pinea* needles, several assays and tests were performed beside the PAH extraction.

The water content of both pine species was determined based on a study of Hubert et al. (2003): three samples of 5 g each were dried at 80 °C until constant weight. The lipid content of the needles was measured also using triplicates of 5 g samples and following the procedure presented by Tomashuk (2008). In brief, dry needles (as after the water content assays) were sonicated for 1 h in 30 ml of hexane:acetone (50:50) and then for another hour with 30 ml of fresh solvent. The extract was then placed in a pre-weighed pear-shaped flask and the solvent was first allowed to evaporate at ambient temperature for 48 h and subsequently rotaevaporated to dryness at 30 °C. The difference in mass of the flask gave the weight of the lipid fraction.

Wettability was determined by measuring the contact angle between a 1 μl droplet of ultrapure water and the adaxial part of the needle surface, in a DataPhysics OCA Series device (San Jose, CA, USA). Four replicates along the length of the needles were considered for this purpose.

Total surface area were determined by mercury porosimetry using a Quantachrome PoreMaster apparatus (Boynton Beach, FL, USA) at 20 °C with a 480 erg cm^{-2} Hg surface tension and a pore diameter range between 3.6 nm and 10.6 μm .

The needles of both pine species were also inspected by light microscopy and scanning electron microscopy (SEM). The first was performed with a Leica DMI 5000 M microscope (Wetzlar, Germany) equipped with a Leica DFC 350 FX camera and numerical aperture of 0.12 for 5 \times magnification. SEM analysis was done in a FEI Quanta 400 FEG ESEM/EDAX Pegasus X4M microscope (Hillsboro, OR, USA) in the high vacuum mode with 15 kV acceleration voltage. The needles (dried at 50 °C for 48 h) were mounted on a brass stub with carbon tape, glued with carbon paint and sputter coated with an alloy of gold and palladium.

2.4. Extraction and quantification of PAHs

The extraction of needle samples (5 g, in duplicate) was performed by sonication and purified with alumina solid-phase extraction (SPE) cartridges, in accordance with a previously reported methodology (Ratola et al., 2006). Chromatographic analysis of PAHs was accomplished by a Varian CP-3800 gas chromatograph (Lake Forest, CA, USA) coupled to a Varian 4000 mass spectrometer in electron impact mode (70 eV) and a CP-8400 auto-sampler. After a 2 μl injection in splitless mode, compound separation was done in a 30 m \times 0.25 mm I.D. DB-5 column coated with 5% diphenyl-polydimethylsiloxane (film thickness 0.25 μm) from J&W Scientific (Folsom, CA, USA). The oven temperature program started at 60 °C, was held for 1 min, then augmented to 175 °C at 6 °C min^{-1} , held for 4 min, increased to 235 °C at 3 °C min^{-1} and finally incremented to 300 °C at 8 °C min^{-1} and kept until a total runtime of 60 min. Injector, transfer line and ion source temperatures were 280, 250 and 200 °C, respectively, and helium (99.9999% purity) was the carrier gas, at 1.0 ml min^{-1} . The acquisition was done in single ion storage (SIS) mode using five retention time windows, each with one deuterated PAH acting as internal standard as reported by Ratola et al. (2009), and PAHs were identified and quantified using retention time and up to three ions with the MS Workstation 6.6 software from Varian. Anthracene- d_{10} was added to all samples just before injection, to detect possible GC–MS errors.

2.5. Method validation (quality assurance/quality control)

Good chromatographic resolution with linear behavior between 0.01 and 1 mg l^{-1} , was obtained for all PAHs

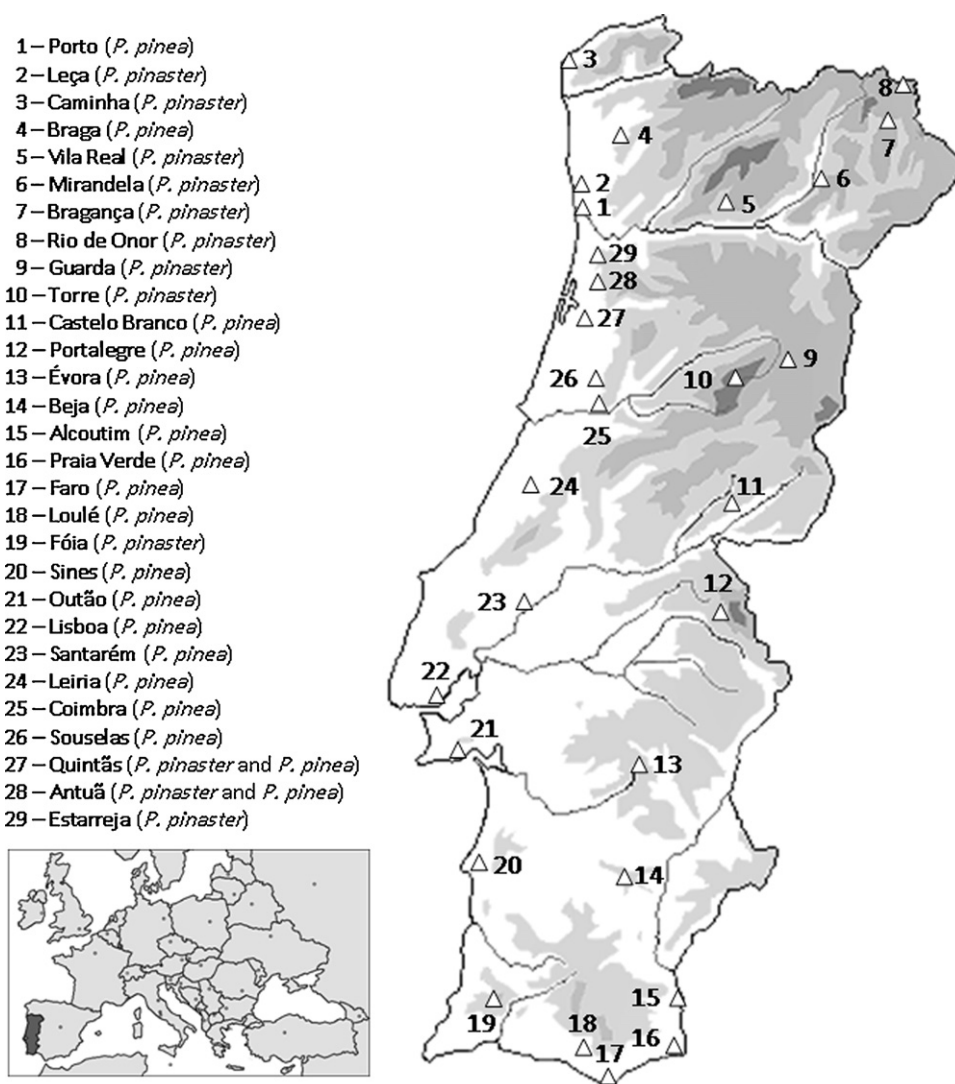


Fig. 1. Sampling map (Portugal).

except for indeno[1,2,3-*cd*]pyrene and dibenzo[*a,h*]anthracene ($0.01\text{--}0.75\text{ mg l}^{-1}$). Limits of detection (LOD) were calculated by the signal-to-noise ratio of 3 rule, using the direct injection of the lowest concentration PAH standard. Values ranged from 0.08 ng g^{-1} (dry weight) for fluoranthene to 1.53 ng g^{-1} (dw) for dibenzo[*a,h*]anthracene, similar to others reported previously for comparable conditions (Martínez et al., 2004; Ratola et al., 2006). The relative standard deviations (RSD%) for two sample duplicates of both species were below 12%, except for naphthalene (16.5%), benzo[*a*]anthracene (14.5%) and indeno[1,2,3-*cd*]pyrene (13.9%) for *P. pinaster* (16.5%) and naphthalene (12.6%) for *P. pinea*. A water content of 59% was found for both *P. pinaster* and *P. pinea* needles.

3. Data analysis

Univariate (*F*-test for analysis of variance; independent two-sample *t*-test for unequal sample sizes and unequal variance and dependent *t*-test for paired samples, for a 95% confidence interval) and multivariate (Principal Component Analysis) data analysis were performed with the obtained concentrations of PAHs.

3.1. Principal Components Analysis

Principal Components Analysis (PCA) is a decomposition technique that aims to extract the relevant and uncorrelated infor-

mation from the original data set. This information is condensed in a few set of Principal Components (PCs) composed by a scores vector (related to the samples) and a loadings vector (related to the variables). It has been widely used in many fields and the fundamentals and data treatment procedures can be found elsewhere (Martens and Næs, 1984; Næs and Martens, 1984; Massart et al., 1997). In the current work, PCA was performed to assess the correlation of a total of 248 pine needle samples (in duplicate) described by the concentration levels of 16 PAHs with biological (pine species) factors. In this case, the whole data set yielded a matrix with 496 row samples and 16 column variables. The variables were autoscaled prior the PCA analysis. Hence, the mean of the column elements was subtracted to the individual elements and divided by the standard deviation of the respective column. Each column has then zero mean and unit variance (Brodnjak-Vončina et al., 2002).

PCA was performed using the PLS Toolbox v. 3.5 (Eigenvector Research, Manson, WA, USA) functions in MATLAB v. 2009b (The Mathworks, Natick, MA, USA) program environment.

4. Results and discussion

4.1. Univariate analysis

Considering all samples analysed regardless of needle year, season and location, the mean total PAH concentration shown by the

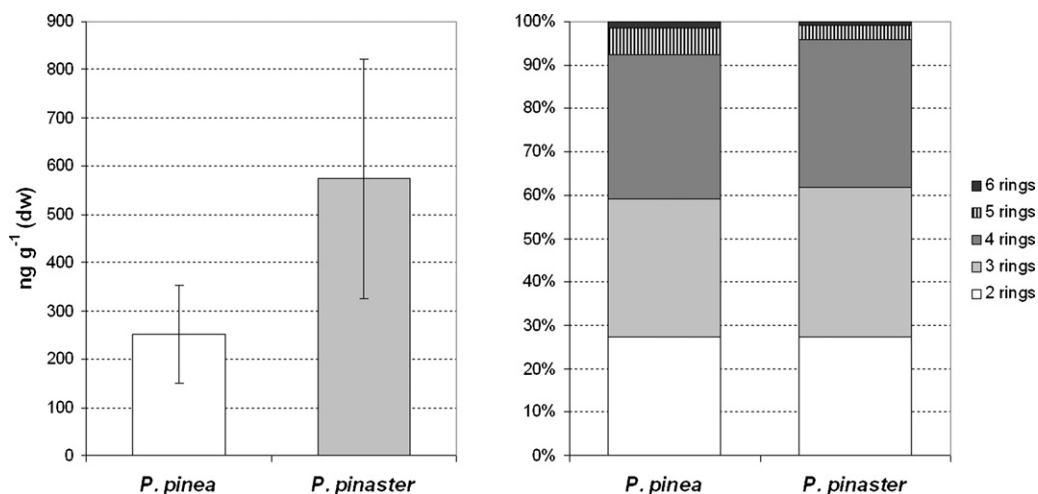


Fig. 2. Comparison of mean total PAH concentration and mean aromatic ring percentage between *Pinus pinea* and *Pinus pinaster*, for all sites and samples.

P. pinaster needles ($574 \pm 248 \text{ ng g}^{-1}$ (dry weight)) is significantly higher than for *P. pinea* needles ($251 \pm 102 \text{ ng g}^{-1}$ (dw)), as seen in Fig. 2. This might reflect diverse entrapment abilities of the two pine species. Overall, the maximum PAH concentration did not reach 800 ng g^{-1} (dw) for *P. pinea* sites and rose up to almost 2000 ng g^{-1} (dw) for *P. pinaster*'s. In another study done in the same sites, Ratola et al. (2010a) found similar concentration discrepancies ($748 \pm 520 \text{ ng g}^{-1}$ (dw) for *P. pinaster* and $399 \pm 197 \text{ ng g}^{-1}$ (dw) for *P. pinea*). Although with a considerably lower number of samples studied, other authors reported some differences. Librando et al. (2002) obtained values between 400 and 1000 ng g^{-1} for *Pinus halepensis* and between 300 and 700 ng g^{-1} for *P. pinea* needles, while Piccardo et al. (2005) reported levels from 12.2 to 507.3 ng g^{-1} (dw) for *Pinus nigra* and from 10.4 to 817.4 ng g^{-1} (dw) for *P. pinaster* samples. These differences have also been reported for other species and several types of plant tissues of the same species, such as in the case of metals uptake (Dunn et al., 1996).

Taking into account the percentage of PAHs incidence by number of aromatic rings (Fig. 2), there are no significant differences among both pine species, except for the 5-ring PAHs, which only contribute with a load less than 7%. The most abundant are the 4-ring PAHs, followed by the 3-ring and the 2-ring. This is in line with the predominant uptake of the lighter PAHs (which are found in the atmosphere in the gaseous phase) by pine needles instead of

the heavier ones, found in the particulate phase (Hwang and Wade, 2008). Considering each PAH individually, the most significant difference is seen for phenanthrene (higher in *P. pinaster*), which is also the most concentrated overall.

If the results are considered by weight of the lipid fraction instead of the dry weight, the differences between species are reduced (which is also suggested by Tian et al. (2008)) but still statistically significant. The amount of wax, essential for the entrapment of organic contaminants, varies with the species, the genotype, the leaf age and the environmental conditions (Pallardy, 2008). Whereas both species have the same percentage of water (59% on average) they differ in the percentage of lipids with *P. pinaster* showing a higher content (7.5%) as compared to *P. pinea* (5.0%), in line with other values presented in the literature (Gaggi et al., 1985). If the weight of the lipid fraction instead of dry weight is taken as reference value the differences between the species decrease but still are significant. The mean total PAH concentrations were $3137 \pm 1358 \text{ ng g}^{-1}$ (lipid weight – lw) and $2056 \pm 833 \text{ ng g}^{-1}$ (lw) for *P. pinaster* and *P. pinea*, respectively. A higher lipid content coincides with a higher PAH content (Simonich and Hites, 1994).

A more accurate comparison is obtained considering only the two sites with two adjacent trees of both species: Antuã (site 28) and Quintãs (site 27). In this case, however, only the needle year

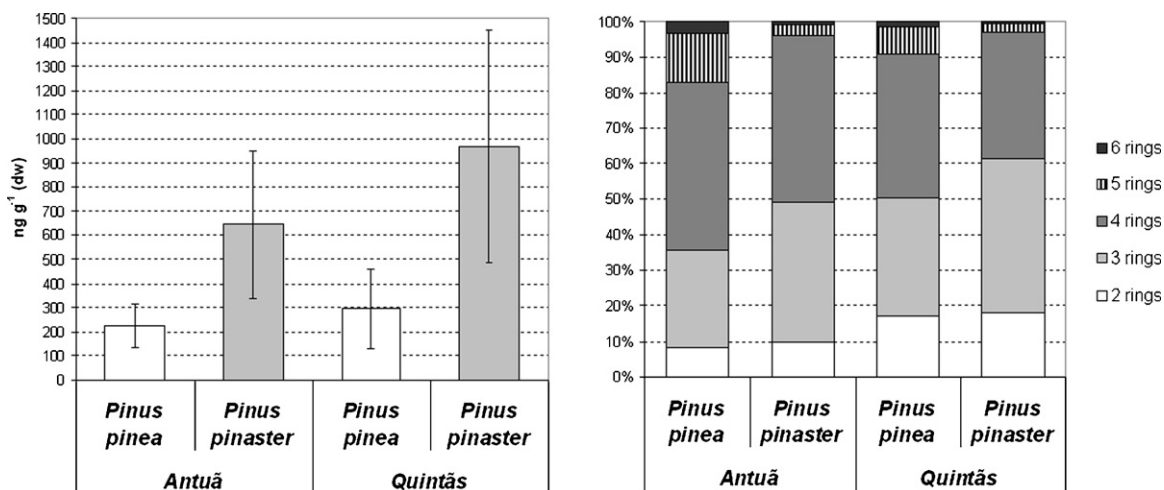


Fig. 3. Comparison of mean total PAH concentration and mean aromatic ring percentage between *Pinus pinea* and *Pinus pinaster*, for all samples collected in Antuã and Quintãs.

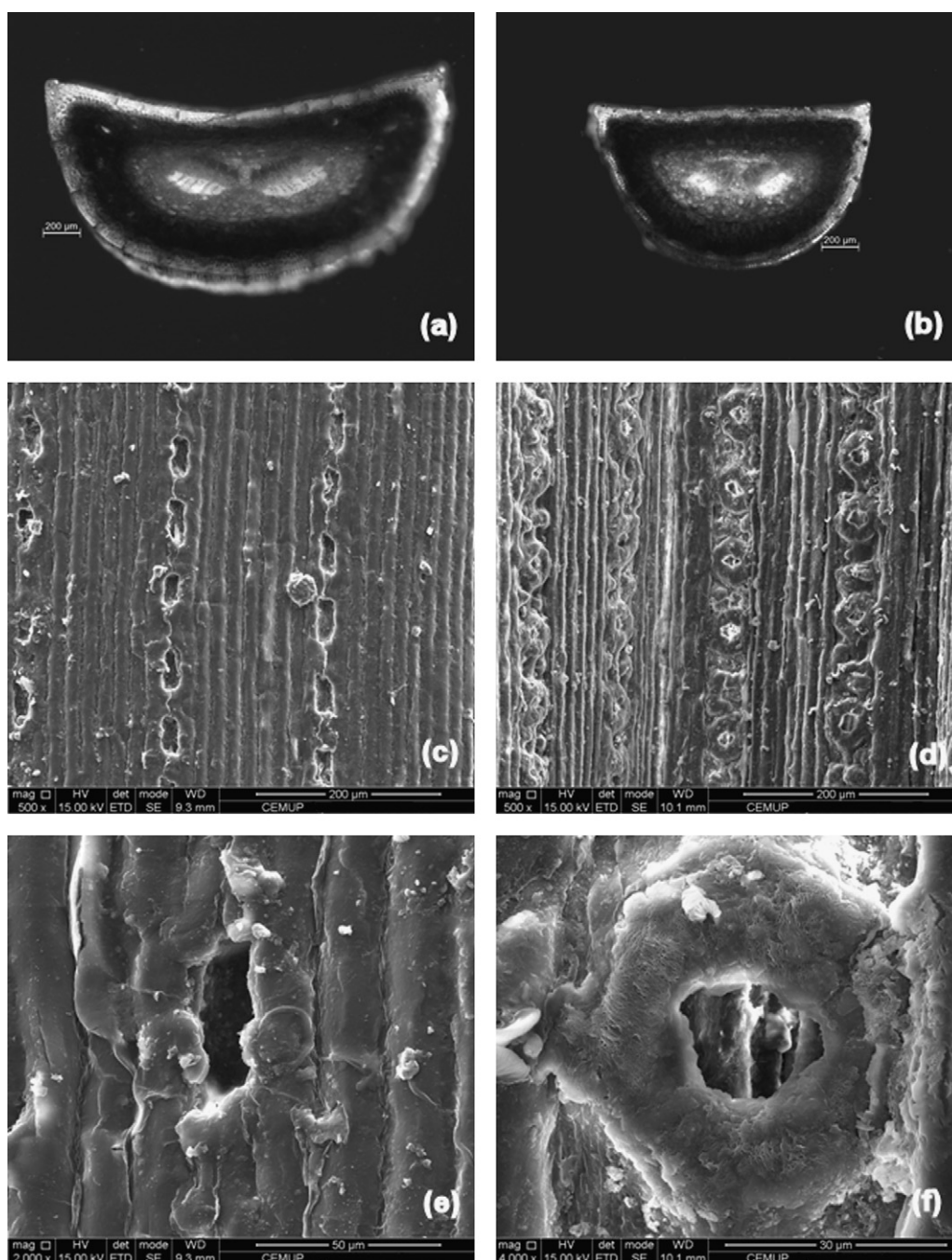


Fig. 4. (a) Transverse section of a *Pinus pinaster* needle by light microscopy (5 \times); (b) transverse section of a *Pinus pinea* needle by light microscopy (5 \times); (c) superficial structure of a *Pinus pinaster* needle using SEM (500 \times); (d) superficial structure of a *Pinus pinea* needle using SEM (500 \times); (e) stoma in a *Pinus pinaster* needles using SEM (2000 \times); (f) stoma in a *Pinus pinea* needle using SEM (4000 \times).

classes available in both species were compared. Since the *P. pinea* needles have a shorter life-span, the 2005 summer and autumn samplings were not considered. Fig. 3 reinforces the previous findings, since the differences between the mean total PAHs of *P. pinea* and *P. pinaster* needles are statistically significant in both sites. Fig. 3 shows the results in dry weight, but the findings for lipid weight are similar, despite the reduction in the mean concentration differences. Regarding the ring patterns, differences between sites are expected since diverse locations may imply diverse pollutant sources. However, there were also significant differences between both species in each site, namely in the percentages of 3-ring (higher for *P. pinaster*) and 5-ring PAHs (higher for *P. pinea*). In Antuã, 6-ring PAHs are also significantly higher for *P. pinea*.

The morphology of the needles in each species may have a crucial role in these distributions. Parameters such as specific leaf area, lipid content and leaf surface roughness are often considered as affecting the concentration of PAHs and other semivolatile organic pollutants in plant leaves (Simonich and Hites, 1995; Franzaring and Eerden, 2000; Moeckel et al., 2008). Although having similar main functions in both pine species, such as the carbon assimilation for the photosynthesis and acting as the reservoir of mineral nutrients (Correia et al., 2007), *P. pinaster* needles are thicker and longer, which favors the entrapment of the lighter gas-phase PAHs into the thicker waxy layer. The light microscopy of the cross-section of the needles (Fig. 4a and b) reveals a semi-cylindrical shape (although more elongated in width for *P. pinaster*) and a very similar dis-

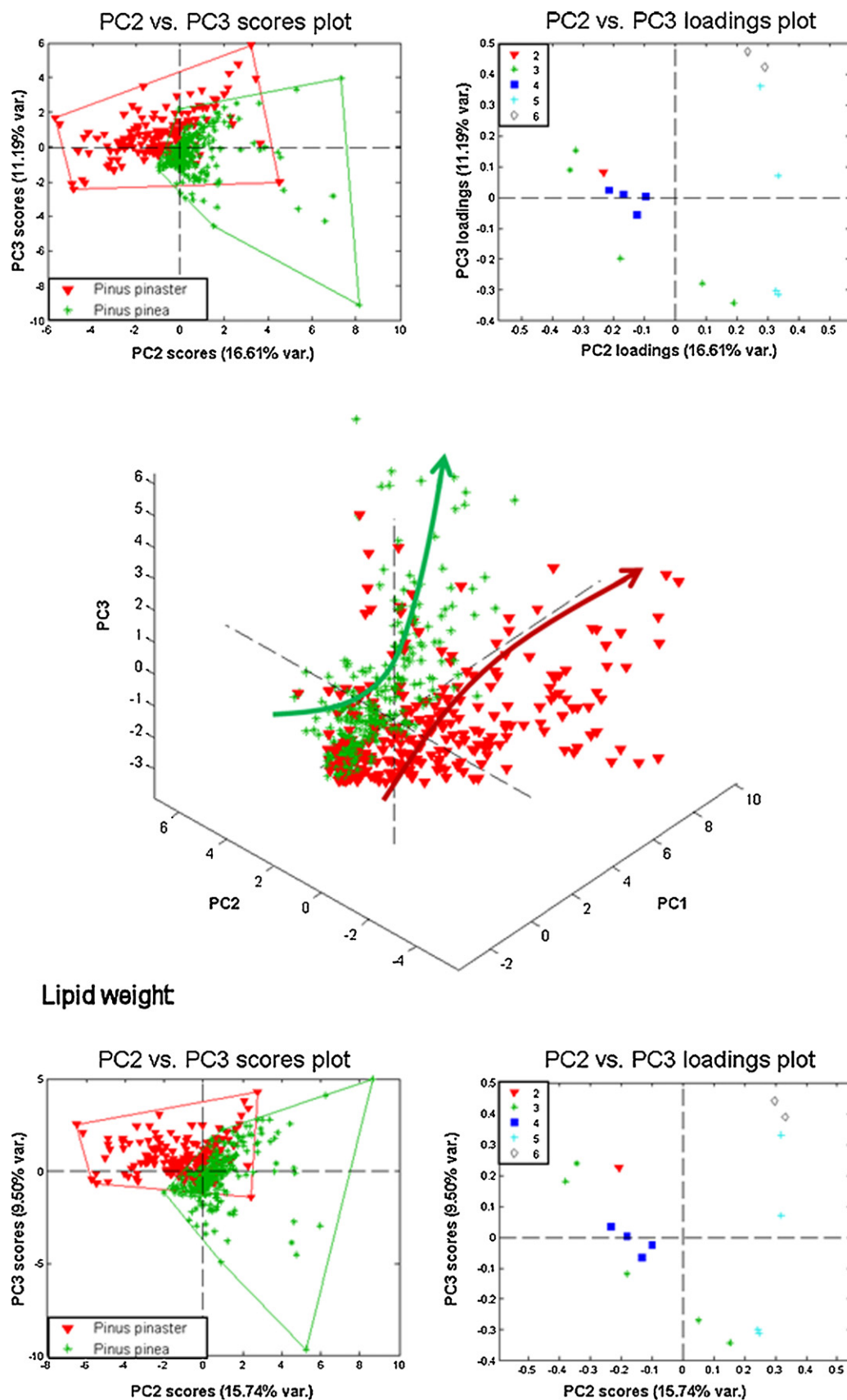


Fig. 5. Scores and loadings plots for new year (2007), 1-year (2006) and 2-year (2005) needles, all seasons, all sites (red inverted triangles – *Pinus pinaster* needles; green stars – *Pinus pinea* needles). Top: PC2 vs. PC3 scores and loadings plots (left and right, respectively) for PAH concentrations in dry weight. Centre: combined scores plot of PC 1, PC 2 and PC 3; bottom: PC2 vs. PC3 scores and loadings plots (left and right, respectively) for PAH concentrations in lipid weight. Labels on loadings plots indicate number of aromatic rings in a molecule of each PAH. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

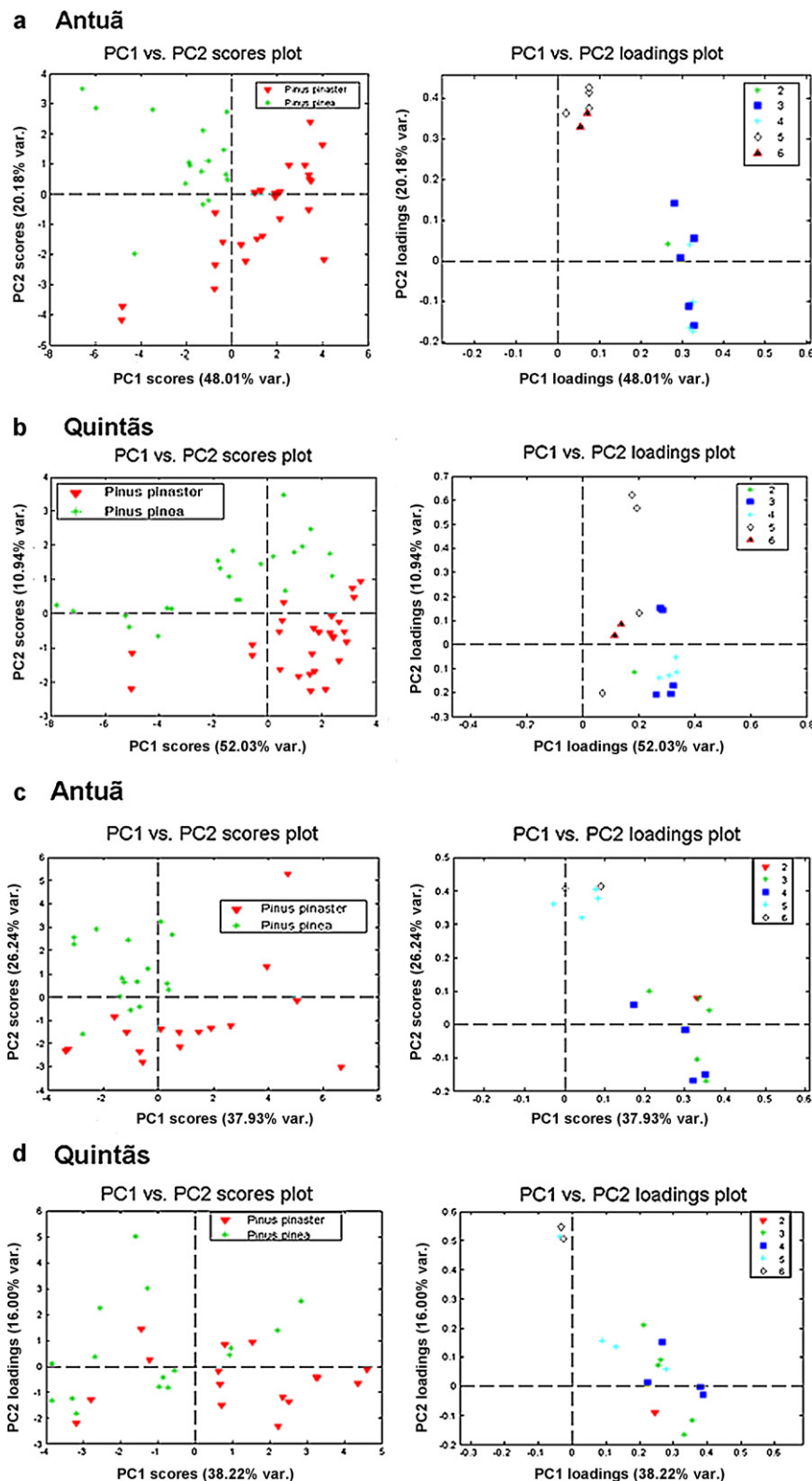


Fig. 6. PC1 vs. PC2 scores (left) and loadings (right) plots for Antuã and Quintãs samples for PAH concentrations in dry weight (a and b, respectively) and lipid weight (c and d, respectively). Labels on loadings plots indicate number of aromatic rings in a molecule of each PAH.

play of the needles inner components (epidermis, stomata, central cylinder, phloem and xylem).

Wettability assays yielded contact angles of $60.7 \pm 2.3^\circ$ for *P. pinaster* and $54.6 \pm 3.9^\circ$ for *P. pinea*, similar to those found by Rettori et al. (2005). This means that *P. pinea* needles are slightly more

wettable, hence favoring the wet deposition of atmospheric pollutants. However, wet deposition accounts only for about 10% of the total atmospheric PAH deposition (McVeety and Hites, 1988). Being less wettable, *P. pinaster* needles tend to have a superior resistance to the establishment of a water layer on the needle surface

which would difficult the entrapment of PAHs by dry deposition. In addition, Cape et al. (1989) reported that the wax quantity of pine and spruce needles decreased with increasing wettability, which may lead to lower entrapment ability towards organic pollutants.

Using SEM analysis (Fig. 4c–f) and considering the conditions used it was possible to find out the main differences in the upper layer structure, which were mainly in the shape and organization of the stomata. The leaf surface of both species is characterized by parallel rows of stomata (less for *P. pinaster* as compared to *P. pinea*) and elongated epidermal cells (Fig. 4c and d). The surface is covered by a layer of epicuticular wax with few crystallites. The epistomatal chamber of *P. pinaster* has an elongated shape and a wide pore diameter (Fig. 4e and f) whereas that of *P. pinea* is smaller and shows a distinct rim (Florin ring). García Álvarez et al. (2009) also reported larger stomatal pore diameter and more homogeneity in the stomatal rows for *P. pinaster*.

Mercury porosimetry assays corroborate these assessments. This technique determines the surface area through the intrusion of a non-wetting liquid (in this case mercury) at high pressure into the pores. The mean surface area of *P. pinea* needles is higher than *P. pinaster*'s (0.76 vs. 0.47 m² g^{−1}), which can suggest a stronger affinity of *P. pinea* towards the particulate-phase contaminants, which tend to rest in the needles surface.

When considering the needles physiology, the number of individual compounds is vast (Roussis et al., 1995; Stabentheiner et al., 2004). Hence, it is virtually impossible to investigate all the variables prone to contribute to the dissimilar behavior of pine needles from different species in one isolated study. This biochemical variability may also account for different loads and mechanisms of pollutants uptake. The importance of the diversity and concentration of starch (Kylin and Sjödin, 2003) and some volatile constituents such as terpenoids (Kylin et al., 2002) have been suggested to influence the uptake of organochlorine pesticides by the needles. For instance, it is known that regarding the monoterpene composition, α -pinene is clearly dominant in *P. pinaster* needles and β -pinene in *P. pinea* instead (Tiberi et al., 1999). In addition, changing needle properties are reported throughout the year (Kylin and Sjödin, 2003) and in different needle years (Stabentheiner et al., 2004).

A larger range of environmental factors (geologic, topographic, hydrologic, edaphic) may influence PAH interspecies differences, but when considering adjacent trees these parameters are more or less leveled. In light of all the evidences, it is clear that care must be taken when considering different species to perform biomonitoring studies, if an accurate comparison is intended.

4.2. Principal Components Analysis interpretation

To assess differences between pine species, the available samples corresponding to the 2005, 2006 and 2007 needle year classes were selected. Since every site except Fóia was represented in all seasons, an accurate and representative comparison was achieved. The scores and loadings plots are presented in Fig. 5 and, at first sight, it can be noticed that the two species are clearly separated in all the plots, where PC 1 accounts for 48% of the variance, PC 2 for 16% and PC 3 for 11%. The tri-dimensional plot (Fig. 5, centre) indicates an overall opposite tendency of the two species. This, in combination with the loadings plot (top right in Fig. 5) is an indication that the PAH concentration patterns in *P. pinaster* needles were significantly different than in *P. pinea* needles. In fact, the loadings plot confirms that *P. pinea* is more prone to the uptake of the heavier PAHs (5 and 6 rings), as suggested earlier by the surface properties of the needles. Furthermore, it confirms the importance of employing a single vegetation species in these kinds of biomonitoring studies whenever possible. If the scores plot is built considering

the lipid weight fraction, the conclusions are similar, as can be seen in the bottom of Fig. 5.

Since these plots comprise all sites, the possible influence of the geographic location is not considered. Therefore, to further clarify data interpretation, a separate analysis has to be done, with the samples from the sites with adjacent trees from both species (Antuã and Quintãs). The PCA of all data available from Antuã (with results in dry weight form) is presented in Fig. 6a. The scores plot reinforces the differences between the two pine species. There is a clear group separation in PC 1 (48% of the variance) and PC 2 (20% of the variance). The corresponding loadings plot demonstrates that the PAHs are perfectly separated by number of aromatic rings, namely the lighter (2–4 rings) which appear in gaseous phase in the atmosphere and the heavier (5 and 6 rings), more prone to the particulate phase. The former, which globally appear in lower concentrations are somehow linked with the *P. pinea* needles, while the most abundant (3 and 4 rings) predominate in *P. pinaster* needles. This also reinforces the descriptive conclusions. Fig. 6b presents the same approach for Quintãs samples (in dry weight). The separation in the scores plot is identical to that of Antuã. In this case PC 1 explains 52% and PC 2 almost 11% of the variance and the difference between rings in PAHs in the loadings plot is not so evident. Nevertheless, the relationship between the higher PAHs and *P. pinea* needles is still visible.

The plots for Antuã and Quintãs with the results in lipid weight form are shown in Fig. 6c and d, respectively. Overall, the separation between pine species is still clear, although not as much in the Quintãs site. However, even in this site, the tendency for *P. pinea* to be correlated with the heavier PAHs is unchanged, as can be seen by the shift of some of the 5-ring PAHs towards the boundary of the PC 2 quadrant in comparison with the dry weight plot. Although most of the biomonitoring studies using pine needles have their results expressed in dry weight, it might be important to take the lipid weight into consideration as well, especially if temporal trends are to be assessed, given the seasonal fluctuations and the ageing processes observed in the wax content of the needles (Turunen and Huttunen, 1996).

It is important to point out that it is plausible that some of these small differences in behavior between the two sites are related to the fact that both sites may be under diverse sources of PAH incidence, due to their specific location. This may favor some PAHs over others, according to their atmospheric presence. Still, the entrapment performance of both pine species is clearly proven.

5. Conclusions

The behavior of the needles of two different pine species (*P. pinaster* Ait. and *P. pinea* L.) in terms of PAH entrapment was found to be dissimilar. Univariate statistics and Principal Component Analysis showed that the mean total PAH concentration of *P. pinaster* needles is significantly higher than *P. pinea* needles and that the former have a stronger affinity towards the lighter PAHs (2–4 aromatic rings) while the latter are more linked to the heavier PAHs (5 and 6 rings). The differences observed in the physical and chemical characterization of the needles both species confirmed these findings. This is an indication that when doing biomonitoring studies with vegetation matrices, it is preferable to use only one species, especially if an accurate comparison is intended between sites.

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